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Contract No. N01-NS-1-2340
Quarterly Progress Report
Oct 1-Dec 31, 2002
Report No.5

"Functional Microstimulation of the Lumbosacral Spinal Cord"

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ABSTRACT

The objective of this project is to develop neuroprostheses that will allow patients with severe spinal cord injuries to regain control of their bladder and bowel. The approach is based on an array of microelectrodes that is implanted into the sacral spinal cord. During the past quarter, an array of 9 discrete iridium microelectrodes was implanted into the sacral spinal cords of cats 4 cats (SP 139, 140, 142 and 143), and 1 array of silicon substrate microelectrodes was implanted into 1 cat (SP 141).

Cat SP138 was sacrificed 84 days after implanting an array into its sacral cord. The array had been implanted using the high-speed introducer, which was adjusted so that the insertion stroke was arrested just before the underside of the array superstructure contracted the spinal pia. This was done to prevent the geometric distortion of the cord during the insertion stroke, which had been observed in the videos of previous insertions, and which we believe to be largely responsible for the gliotic scars that often occur ventral and medial to the microelectrode tips. The precaution used in cat SP138 did indeed results in much less tissue injury ventral to the electrode tips sites. However, the array did not insert to its full depth. We presume that the incomplete insertion could be attributed to dimpling of the spinal pia during the insertion. We will attempt to remedy the problem by decreasing the diameter of the microelectrodes (to 50 µm, from the present 75 µm) and by increasing the velocity at which the electrodes are inserted.

Probably because of the dorsal position of most of the microelectrode tips, the intraspinal microstimulation did not produce a large elevation in bladder pressure or relaxation of the urethral sphincter. However, microstimulation via several of the microelectrodes did produce marked inhibition of spontaneous bladder contractions at 100 µA, 400 µs/phase, at a pulse rate of 20 Hz. The tips of these microelectrodes were in, or immediately adjacent to, the dorsal part of the dorsal horn.

METHODS

The objective of this project is to develop neural prostheses that will allow patients with severe spinal cord injuries to regain control of their bladder and bowel. The system is based on an array of microelectrodes that is implanted into the sacral spinal cord. The procedures and hardware are being developed in cats with intact spinal cords, and in the 2nd year of the project, the cats with functionally stable implants will undergo transection of the spinal cord at the low thoracic level.

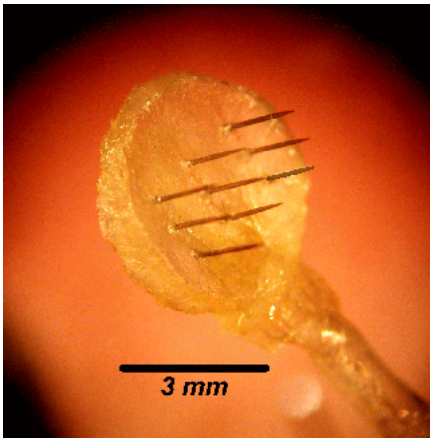

Figure 1

<u>Fabrication and implantation of the microelectrode arrays</u>

Discrete iridium microelectrodes with exposed surface areas of approximately 2,000 μm^2 were fabricated as described previously. The arrays of 9 iridium microelectrodes extend from an epoxy superstructure (Figure 1). In cat SP138, the 3 microelectrodes comprising the middle row were 1.4 mm in length, and those in the outboard rows were 1.5 mm in length. The middle and outboard rows are separated by 0.8 mm, and the electrodes in each row (rostral-caudal separation) is 0.5 mm.

The sacral cord is exposed using a standard dorsal laminectomy. To locate the junction of the S_1 and S_2 segments, we stimulate the perigenital skin, which is innervated from the S_2 dorsal root, and record at intervals of 2 mm along the rostral-caudal dimension on the dorsal surface of the sacral cord. The arrays are implanted near the maximum of the 2nd component of the evoked response (the dorsal cord potential). At autopsy, the position of the array was validated by complete dissection of the spinal roots.

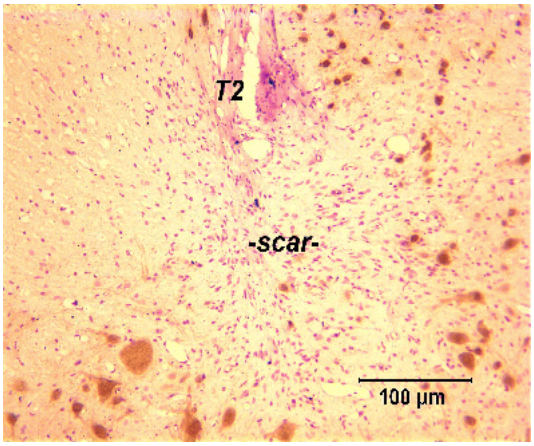

Figure 2..SP135. Tip site of sharp microelectrode #2, with a 250 µm diameter glial scar ventral to the tips site. NeuN + Nissl counterstain.

Previously, we have reported the frequent occurrence of small scars extending ventral and medial to the electrode tip sites. A particularly graphic example is shown in Figure 2. We have attributed this scarring to transient mechanical deformation of the spinal cord during implantation of the array. Therefore, in cat SP138, we adjusted the inserter tool so that during insertion, the underside of the array matrix would not be ejected beyond the end of the barrel of the inserter tool and thus would not impact upon the surface of the cord. A frame-by frame analysis of the intra operative video revealed that there was little displacement of the spinal cord, and as discussed below, there was little or no scarring ventral to the electrode sites. However, the modified insertion

procedure did introduce another problem, namely that the electrodes were not fully inserted.

The microelectrodes in cat SP138 were not subjected to prolonged pulsing, since our objective was to evaluate the modified implantation procedure, independent of any tissue change that might accrue during prolonged stimulation.

Measurements of bladder and sphincter pressure

The cat was lightly anesthetized with Propofol and the urinary bladder was catheterized. Hydrostatic pressure within the bladder vesicle was measured with a transducer, and the digitized data stored in a computer, as described in previous reports. The bladder was evacuated, then filled with sterile saline by elevating the open-top reservoir to a height of 20 to 50 cm above the bladder. The constrictive force within the urethral sphincter was measured as an "infusion pressure"; the resistance to the infusion of saline through a port on the side of the catheter as saline was infused continuously at a rate of 100 ml/hr.

Histologic Procedures

Cat SP138 was sacrificed 84 days after implanting the array. The cat was deeply anesthetized with pentobarbital, given i.v. heparin and perfused through the aorta for 30 seconds with a prewash solution consisting of phosphate-buffered saline, and 0.05% procaine HCl. This was followed by a fixative containing 4% formalin prepared freshly from paraformaldehyde in 0.1 M sodium phosphate buffer. Tissue blocks containing both the microelectrodes and areas rostral and caudal to the electrodes were washed overnight in 4% formalin, then in distilled water for 2 hrs, dehydrated in a graded series of ethanol and embedded in paraffin. The paraffin-embedded tissue blocks were cut at a thickness of 6-7 μm and were picked up on histogrip-coated-slides (4 sections per slide). Electrode tips were located in unstained sections. The slides containing the electrode tips were stained for the neuron-specific protein NeuN, with cresyl violet (Nissl) counterstain or with Nissl alone, in an alternating pattern. This allowed each electrode tip to be evaluated using both stains. Histologic sections were photographed using a SPOT Insight digital microscope camera with 1600 x 1200 pixel resolution.

RESULTS

Figure 3 shows the electrode array after implantation into the caudal S_1 spinal cord of cat SP138, before closure of the dura over the array. The array matrix is slightly elevated above the pia, indicating that the microelectrodes were not inserted to their full depths. We had assumed that when the dura was drawn over the top of the array's superstructure, the array would be pushed down to its full depth, with the underside of the superstructure in contact with the pia. However, as noted below, this did not occur.

Figures 4-8 show histologic sections through the tip sites, or through the sheath surrounding the tip sites of several of the microelectrodes. All of the tips sites were rather shallow

in the cord, due to the failure of the array to insert fully. This was compounded by the fact that the array was inserted into caudal S_1, where the cord is larger, rather than into rostral S_2. There is no glial scarring ventral to the tip sites, and there is no discernible reduction in the density of the NeuN-positive neurons in the region surrounding the tip sites.

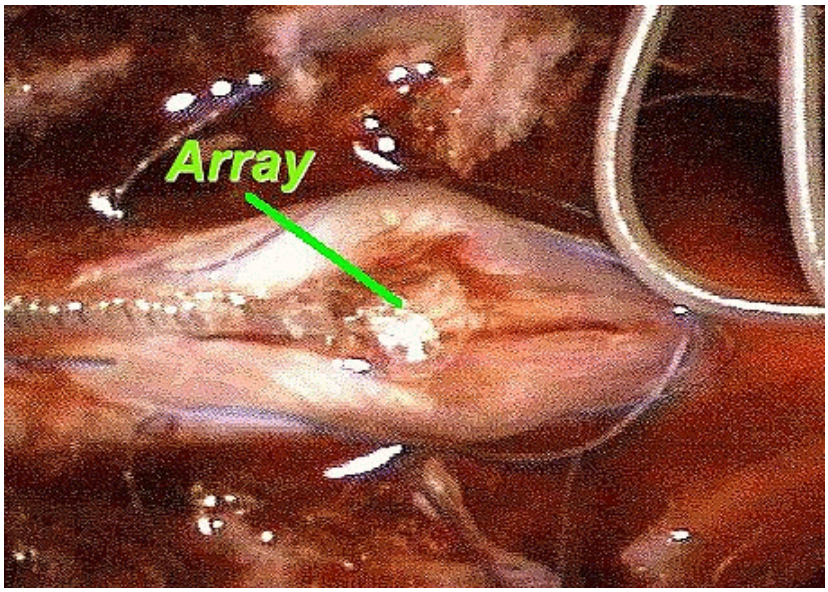

Figure 3. The microelectrode array in the sacral cord of cat SP138, before closure of the dura.

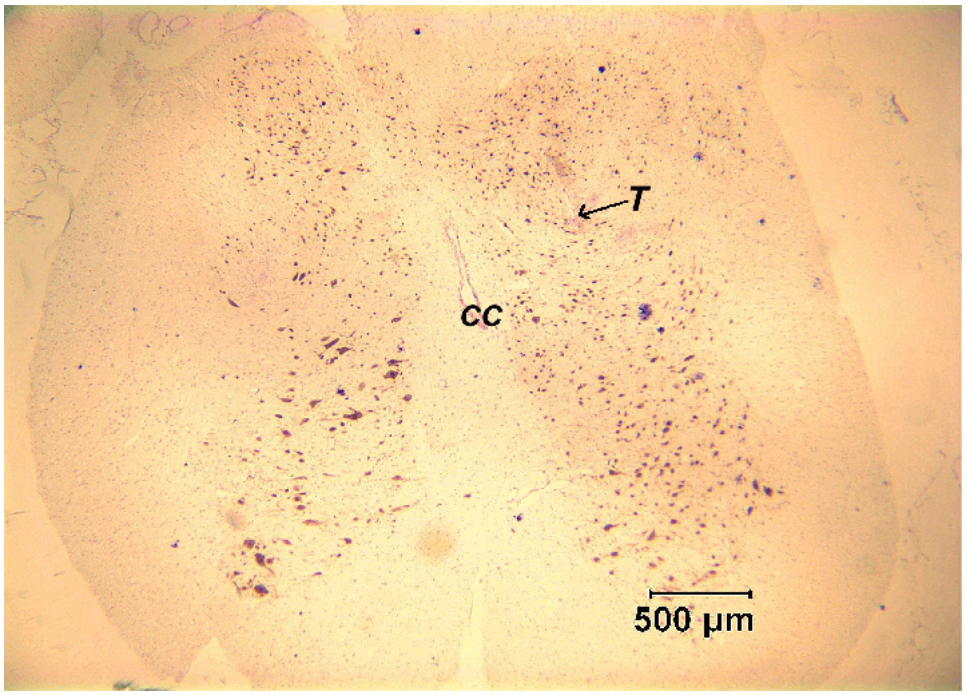

Figure 4A. Low-power view of the S_1 cord shows the tip site (T→) of electrode #1 located dorsomedial to the left intermediolateral cell column. The central canal (CC) is also shown. Immunohistochemical reaction for NeuN, cresyl violet (Nissl) counter stained.

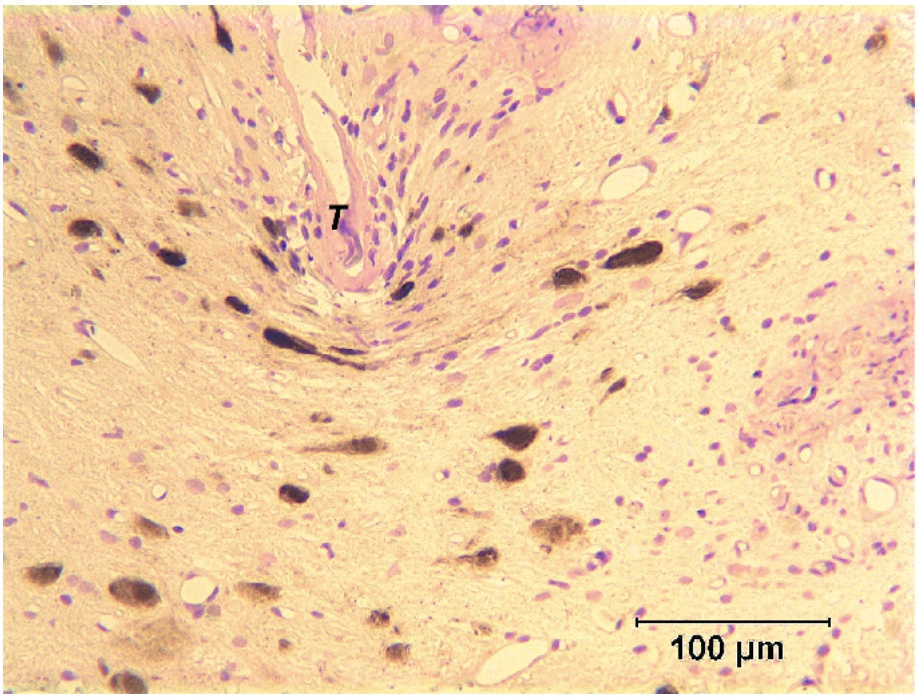

Figure 4B. Higher magnification of the tip (T) of electrode #1 shown in Figure ***A***. Note several brown-colored NeuN-positive neurons within 100 µm of the electrode tip. There is Little gliosis or scarring ventral to the electrode tip. Immunohistochemical reaction for NeuN, Nissl counter stained.

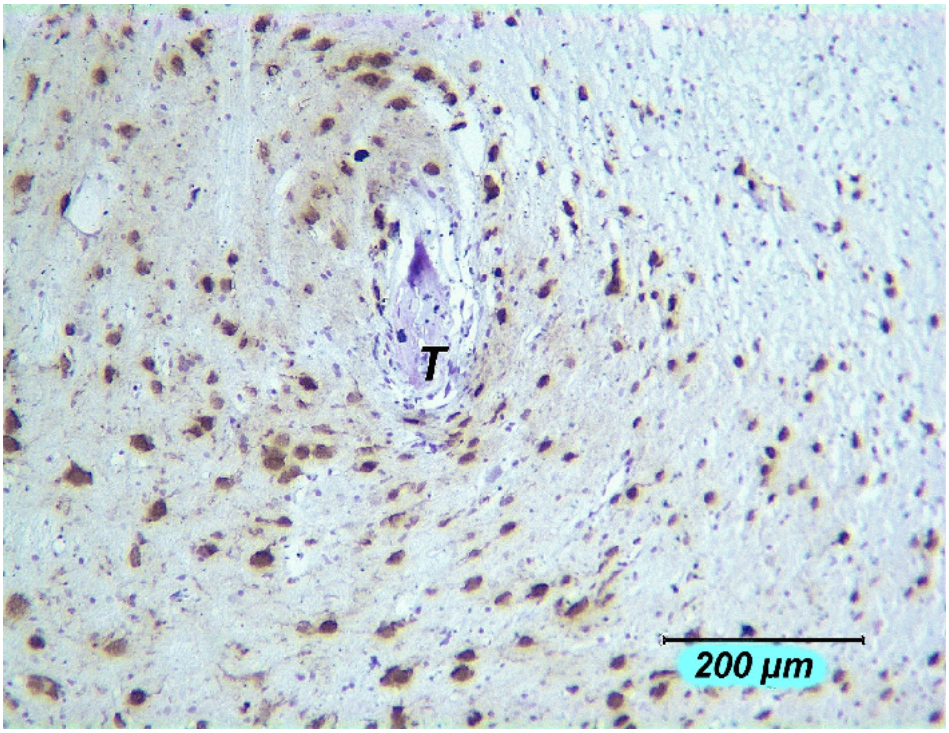

Figure 5A. High magnification of a section adjacent to the tip (T) of electrode #3. Note the brown-colored NeuN-positive neurons close of the electrode tip, and minimal gliosis or scarring ventral to the electrode tip. Immunohistochemical reaction for NeuN, Nissl counter stained.

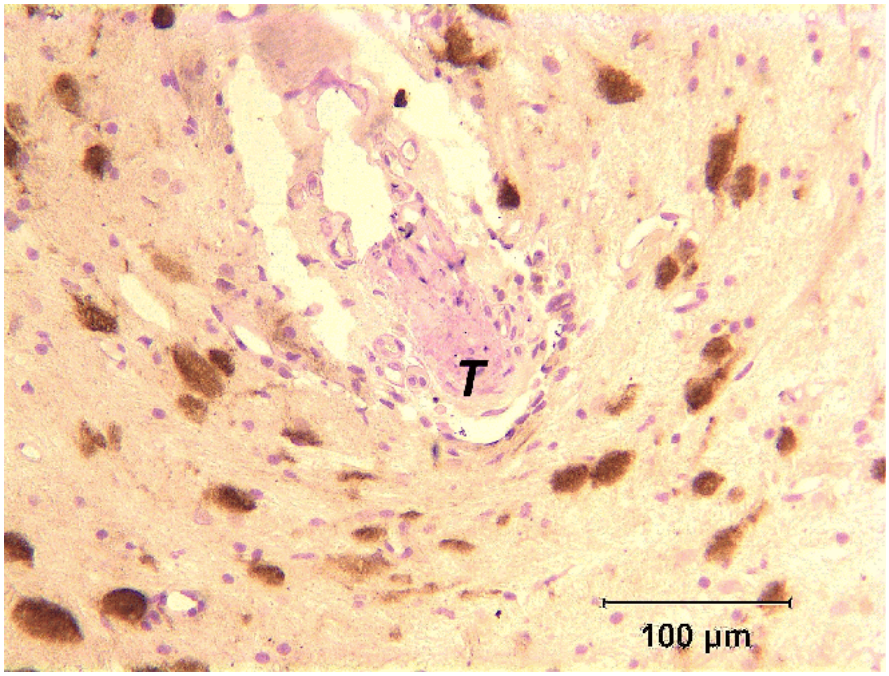

Figure 5B. High magnification of a section adjacent to the tip (T) of electrode #6. The section traverses the capsule surrounding the electrode track and tip. However, there is minimal gliosis and neovascularization near the electrode tip, and there are NeuN-positive neurons close of the electrode tip. Immunohistochemical reaction for NeuN, Nissl counter stained.

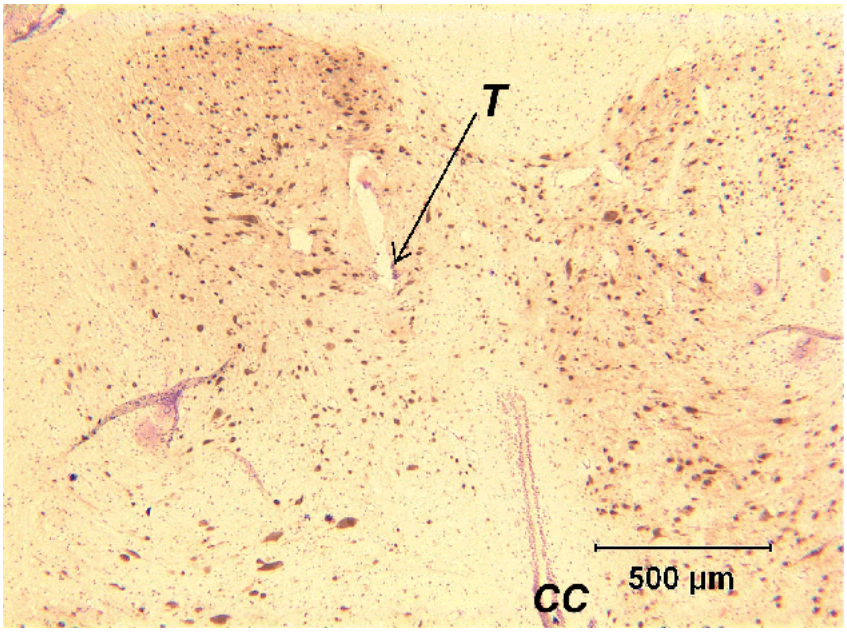

Figure 6A. Low magnification view of the S_1 cord shows the track and the tip site of electrode #7 (T→) dorsal to the dorsal gray commissure. Note the brown-colored NeuN-positive neurons in close proximity to the electrode tip. Immunohistochemical reaction for NeuN, Nissl counter stained.

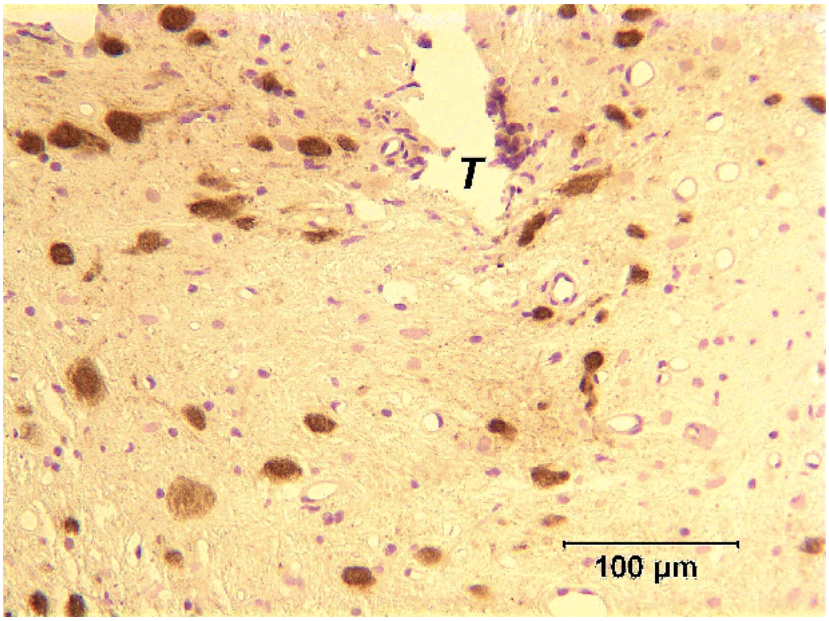

Figure 6B. Higher magnification of the tip (T) of electrode #7. Note several NeuN-positive neurons adjacent to the electrode tip. Little gliosis and a few scattered leukocytes are close to the site of the electrode tip. Immunohistochemical reaction for NeuN, Nissl counter stain.

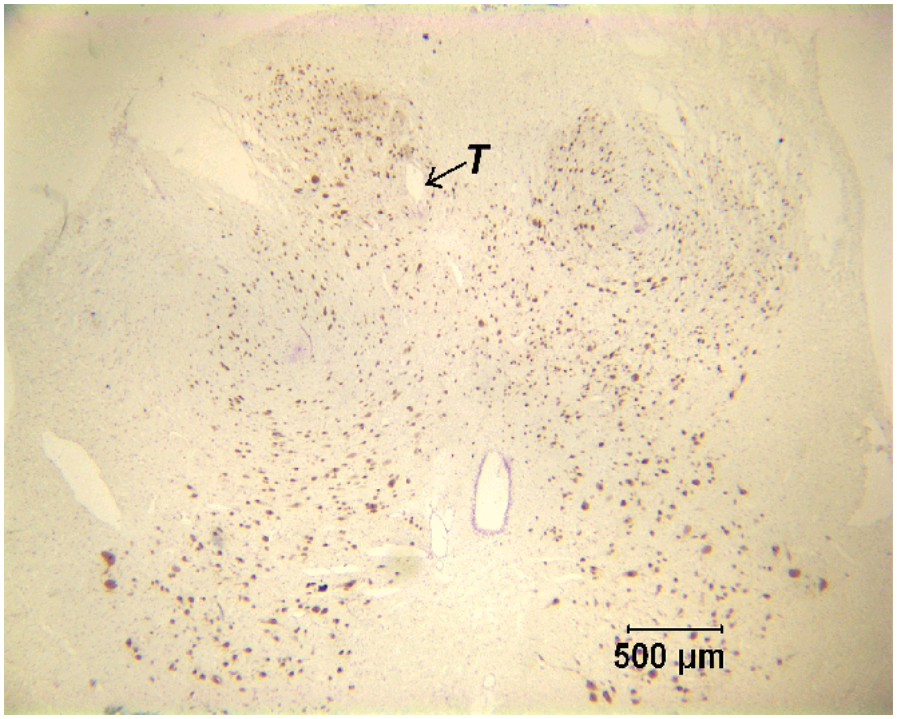

Figure 7A. The tip site (T) of electrode #8, at the medial edge of the dorsal horn. NeuN immunoreaction, Nissl counter stain.

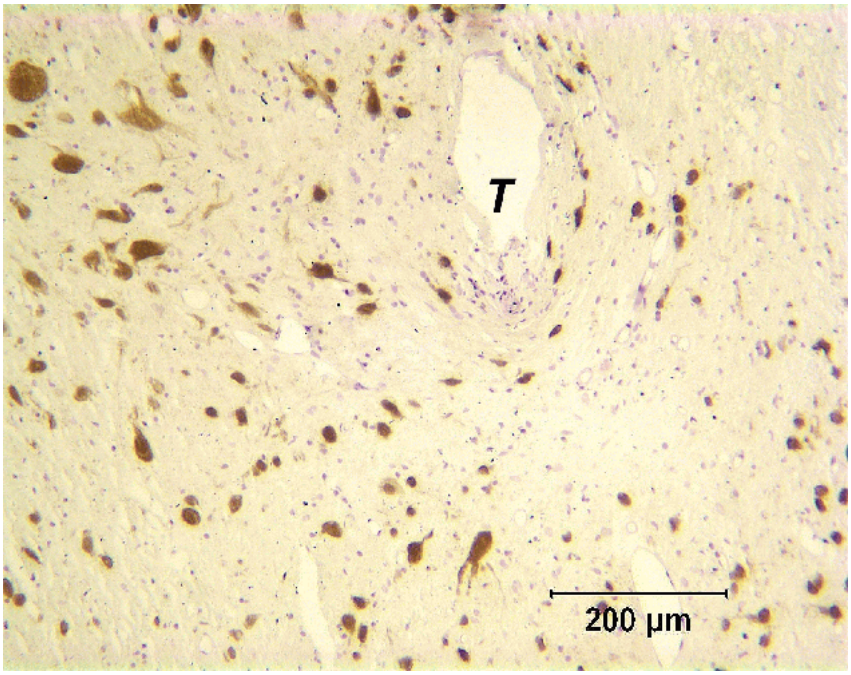

Figure 7B. Higher magnification view of the tip site (T) of electrode #8. Note the NeuN-positive neurons close to the electrode tip. NeuN immunoreaction, Nissl counter stain.

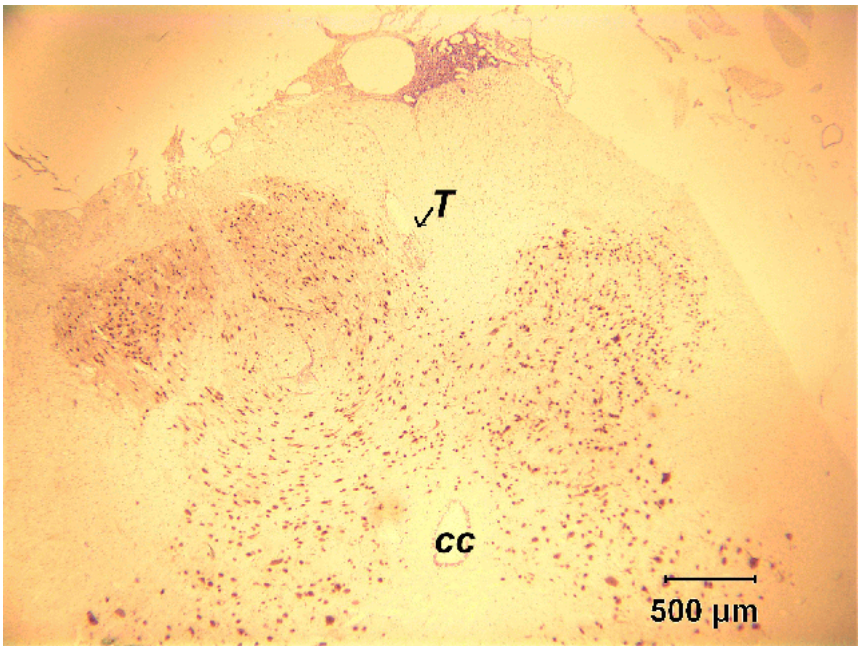

Figure 8A. The tip site (T) of electrode #9, at the medial edge of the dorsal horn. NeuN immunoreaction, Nissl counter stain.

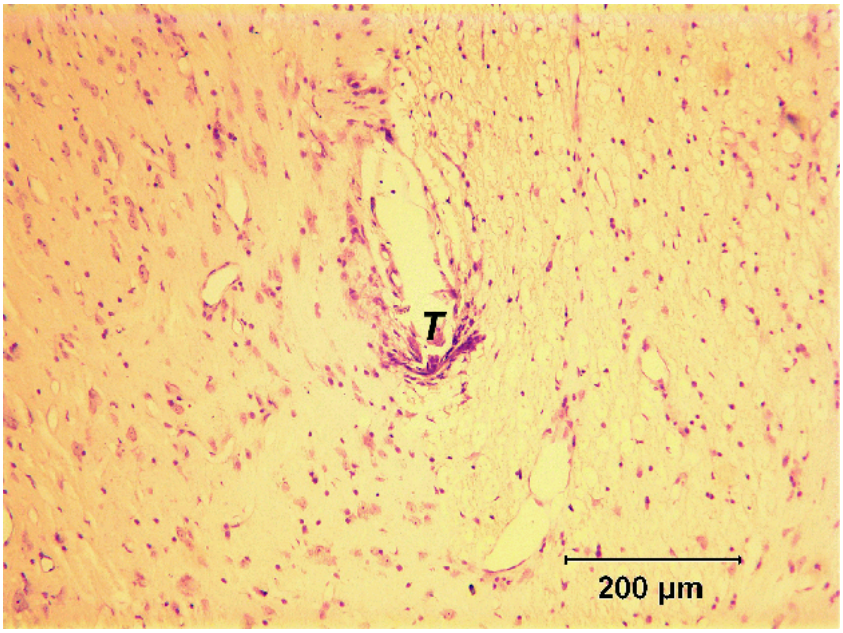

Figure 8B. Higher magnification view of the tip site (T) of electrode #9. Note the NeuN-positive neurons close to the electrode tip. Nissl stain.

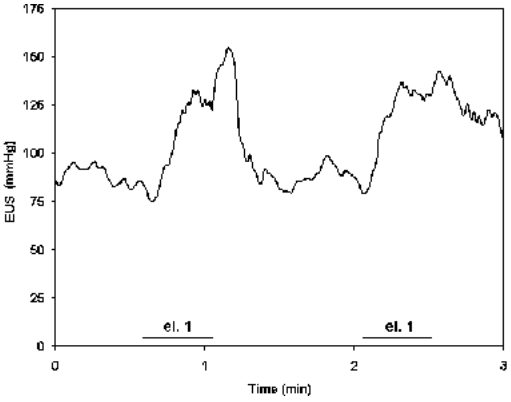

Figure 9. The elevation in the infusion pressure within the EUS produced by stimulating with microelectrode 5

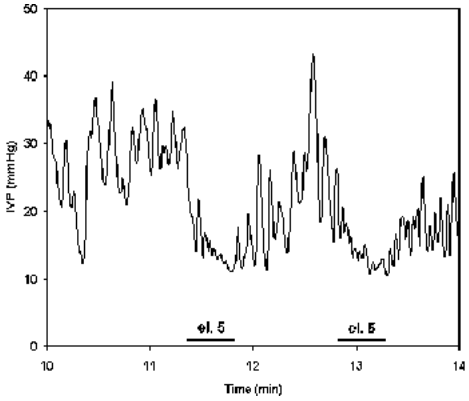

Figure 10. The effect on spontaneous bladder contractions produced by stimulating with microelectrode 5

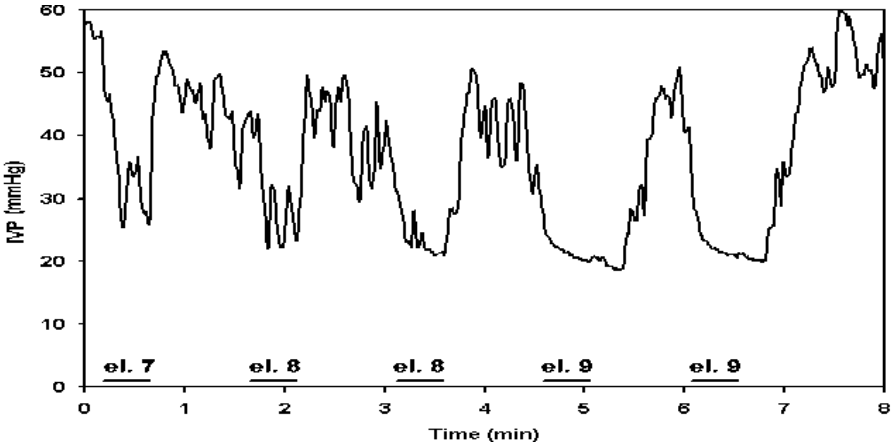

Figure 11. The effect on spontaneous bladder contractions produced by stimulating with microelectrode 7,8 or 9

Reduction of spontaneous bladder activity by the intraspinal microstimulation

Probably because of the dorsal position of most of the microelectrode tips, the intraspinal microstimulation did not produce an elevation in bladder pressure, and none produced complete relaxation of the urethral sphincter. In fact, microelectrode 5 induced an increase in the tonus of the EUS (Figure 9). However, several of the microelectrodes did induce marked inhibition of spontaneous bladder contractions. Using an infusion pump, the bladder was slowly filled (2 ml/min) with warm saline until the bladder pressure reached 25-30 mm Hg. At this pressure, the bladder began to exhibit spontaneous hyperreflexia-like contractions. Bladder contractions were strongly inhibited by intraspinal microstimulation with microelectrodes 5, 7,8 or 9, at 100 µA, 400 µs/phase, at a pulse rate of 20 Hz (Figure 10,11). Microelectrode 9 was most effective and microelectrode 7 was less effective. The tips of all three microelectrodes were in the medial part of the dorsal horn (Figure 6A, 7A, 8A). Of the three, the tip of microelectrode #9 was the most dorsal and that of #7 was the most ventral.

DISCUSSION

We have often reported gliotic scars ventral and medial to the tips sites of the chronically-implanted intraspinal microelectrodes (e.g, figure 2). While these scars do not appear to have significantly compromised the functionality of the implants or inflicted neurological impairment upon the cats, it is certainly desirable to minimize tissue injury due to the microelectrodes. In the last report (QPR #4), we reported that the severity of the scaring was not affected by the sharpness (radius of curvature) of the electrode tips.

The array was implanted into cat SP138 using the high-speed introducer, which was adjusted so that the insertion stroke was arrested just before the underside of the array superstructure contracted the spinal pia. This was done to prevent the geometric distortion of the cord which had been observed in the frame-by-frame examinations of the video tapes of previous insertions, and which we believe to be largely responsible for the tissue injury and subsequent scarring. The precaution used in cat SP138 did indeed results in much less tissue injury ventral to the electrode tips sites. However, the modification of the insertion procedure introduced another problem; the array did not insert to its full depth. We presume that the incomplete insertion could be attributed to dimpling of the spinal pia during the insertion, and to the mobile sacral cord being slightly displaced during the insertion. We will attempt to remedy this problem by deceasing the diameter of the microelectrodes (to 50 µm, from the present 75 µm) and by increasing the velocity at which the electrodes are inserted.

Microstimulation via several of the microelectrodes at 100 µA, 400 µs/phase, at a pulse rate of 20 Hz did produce marked inhibition of spontaneous bladder contractions. The tips of these microelectrodes were in, or immediately adjacent to, the dorsal part of the dorsal horn, which confirms our earlier findings regarding the placement of microelectrodes that can elicit such inhibition (QPR #2). The ability to inhibit spontaneous hyperactivity of the bladder is an ancillary, but important, function of a prosthesis to restore control of bladder to patients with spinal cord

injury. However, it is important that the electrodes be placed optimally (e.g, microelectrode #9), since unlike periodic micturition, suppression of hyperactivity may require nearly continuous stimulation, and the stimulus amplitude must be relatively low in order to minimize the risk of stimulation-induced tissue injury.

Publications & Presentations

The following manuscript was submitted to the Annals of Biomedical Engineering:

McCreery, D. B., Lossinsky, A. S., Pikov, V., Bullara, L. A., and Agnew, W. F. (2003). Chronically Implantable Electrode Arrays for Functional Microstimulation of the Lumbosacral Spinal Cord, submitted to: Annals of Biomedical Engineering.

ABSTRACT:

The objective of this project has been to develop neural prostheses that will allow patients with spinal cord injuries to regain control of their bladder and bowel, using an array of microelectrodes implanted into the sacral spinal cord. For our chronic cat model, we have developed two microelectrode arrays, one type containing 9 discrete activated iridium microelectrodes, and the second type incorporates silicon substrate probes with multiple electrode sites on each probe. We have implanted the arrays of discrete iridium microelectrodes into the sacral cords of 31 cats, and arrays of silicon-substrate probes into 4 additional cats, for up to 144 days. Both array types can elicit an increase in the pressure within the urinary bladder of more than 40 mm Hg and simultaneous relaxation of the urethral sphincter when the microelectrodes are implanted into or near the intermediolateral cell column, at the S_2 or caudal S_1 levels.

A stimulus of 100 μA and 400 μs/ph at 20 Hz (charge-balanced pulses) was required to induce a large increase in bladder pressure or relaxation of the urethral sphincter. We found that 24 hours of stimulation at 100 μA and 400 μs/phase induced tissue injury (disrupted neuropil, infiltration of inflammatory cells, and loss of neurons close to the tip sites). However, a neural prosthesis that is intended to restore bladder control after spinal cord injury would not operate continuously. Thus, when this stimulus is applied as a 10% duty cycle (1 minute of stimulation, 9 minutes without stimulation) only minimal histologic changes were observed.

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We also presented some of these results as a poster at the 32nd Soc. For Neurosciences meeting: Lossinsky, A.S., D.B. McCreery, L.A. Bullara, V. Pikov, and W.F. Agnew. Immunohistochemical and histochemical examination of neuronal changes after electrical stimulation of the feline sacral spinal cord. *Abstract & poster presented at 32nd Meeting of the Soc. for Neuroscience, Orlando, FL*, 2002.